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Review

A Protein Phosphatase 2A-Based Assay to Detect Okadaic Acids and Microcystins

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Abstract: Okadaic acids (OAs) are causative agents of diarrhetic shellfish poisoning, produced by the dinoflagellates *Dinophysis* spp. and *Prorocentrum* spp. Microcystins (MCs) are cyclic heptapeptide hepatotoxins produced by some cyanobacteria genera, including *Microcystis* spp. Traditionally, toxicity detection and quantification of these natural toxins were performed using a mouse bioassay (MBA); however, this is no longer widely employed owing to its lack of accuracy, sensitivity, and with regard to animal welfare. Therefore, alternative toxicity analyses have been developed based on MCs' and OAs' specific inhibition of protein phosphatase 2A (PP2A), using *p*-nitrophenylphosphate (*p*-NPP) as a substrate. The assay is simple, inexpensive, ready for use on site, and can be applied to several samples at once. For OA detection, this assay method is appropriate for widespread application as a substitute for MBA, as evidenced by its alignment with the oral toxicity of MBA. In this review, we summarize the structure and function of PP2A, the inhibitory activities of OAs and MCs against PP2A, and the practical applications of the PP2A assay, with the aim of improving understanding of the PP2A assay as an OAs and MCs detection and quantification method, as well as its suitability for screening before confirmatory chemical analysis.

Keywords: protein phosphatase 2A(PP2A); diarrhetic shellfish poisoning (DSP); okadaic acids (OAs); microcystins (MCs); PP2A inhibition assay

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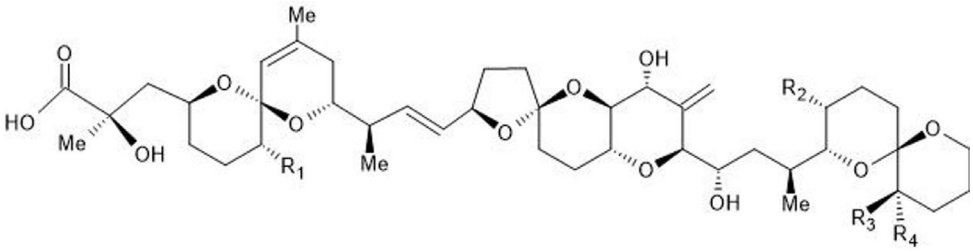
1. Introduction

Marine natural toxins are causative agents of seafood poisoning, including pufferfish poisoning (PFP), ciguatera fish poisoning (CFP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), and paralytic shellfish poisoning (PSP). The chemical structures and biological functions of these toxins have already been described in detail [1]. Monitoring of these toxins in seafood was traditionally performed using a mouse bioassay (MBA) based on lethality from intraperitoneal (i.p.) injection, and toxicities were explained in mouse units. In addition, oral administration was employed to assess the toxicities of each analog. Concerns for animal welfare and requirements for high accuracy, sensitivity, and rapidity fueled a shift toward alternative methods. Various analytical methods, including structure-based methods such as instrumental analysis using liquid chromatography–mass spectrometry (LC-MS) [2–6] and an enzyme-linked immunosorbent assay (ELISA) [7–11], functional assays such as cell-based assays measuring cytotoxicity [12–18], and a protein phosphatase (PP) inhibition assay [11,19–25], have been developed to detect okadaic acids (OAs), which are responsible for DSP, and microcystins (MCs), hepatotoxins in the form of cyclic heptapeptides generated by cyanobacteria. In this review, we focus on the PP inhibition assay, which is simple, cost-effective, field-ready, and capable of handling several samples simultaneously. PPs comprise serine/threonine and tyrosine phosphatase families. PP2A is a representative serine/threonine phosphatase in eukaryotes, from yeast to mammalian cells [26–29]. Reversible protein phosphorylation, controlled by protein kinases and

phosphatases, plays a major role in the regulatory mechanism of cellular activities in eukaryotic cells. PP2A plays pivotal roles in cellular processes such as metabolism, transcription, translation, cell cycle, and signal transduction. Thus, the interception of the control functions of PP2A activity has been linked to diseases such as cancer [30–32] and neurodegenerative disorders [33,34]. OAs and MCs are the best studied phosphatase inhibitors, specifically binding to PP2A and strongly inhibiting its activity [19,35–37]. A colorimetric assay for inhibiting PP2A, relying on its specific inhibitory effect, was suggested for the detection of OAs and MCs, utilizing *p*-nitrophenylphosphate (*p*-NPP) as a substrate [11,20,22–24,38,39]. PP2A hydrolyzes *p*-NPP, a colorless artificial substrate, producing *p*-NP (*p*-nitrophenol), which is yellowish. The color intensity is proportional to the enzyme activity, and its absorbance is measured at 405 nm. The concentration of these toxins in the samples can be calculated from a calibration curve prepared using reference solutions of the toxins. Several other natural toxins and antibiotics have also been demonstrated to inhibit PP2A, e.g., cantharidin [40], calyculin-A [41], fostriecin [42], tautomycin [43], and nodularin [44]. Thus, the PP2A inhibition assay could be a valuable tool for detecting these toxins. This review focuses on the potential of the PP2A inhibition assay as a detection and quantification method for OA group and MC and its variants (MCs), as well as its suitability for screening before confirmatory chemical analysis.

2. Okadaic Acids as Causative Agents of DSP

DSP is a gastrointestinal disease caused by ingesting bivalves (e.g., scallops, mussels, oysters, short-necked clams) containing lipophilic toxins produced by dinoflagellate, *Dinophysis* spp., and *Prorocentrum* spp. [45–47]. Its representative component is OA, a carboxylic acid with multiple ether rings (molecular formula: $C_{44}H_{68}O_{13}$), and its analogs, dinophysistoxins (DTXs). Collectively, they are known as OAs because of their structural similarities: OA, dinophysistoxin-1 (DTX1 = 35-*R*-methyl OA), dinophysistoxin-2 (DTX2 = 31-demethyl-35-*S*-methyl OA), and their 7-O-acyl esters (dinophysistoxin-3: DTX3) (Figure 1). Regarding the origin of the toxins, it was initially demonstrated that the planktonic dinoflagellate *Dinophysis fortii* and seven related Dinophysis species were capable of producing OA and DTX1 [46,48], followed by the confirmation of DTX2 in *Dinophysis acuta* [45]. DTX3 is a group of metabolites in bivalves that are esterified forms of OA, DTX1, and DTX2 at C7-OH with fatty acids [49–51]. While the toxicity of DTX3 is less than that of the parent compounds, it has the potential to undergo hydrolysis by lipases and other enzymes, thereby releasing free-form OAs into the gastrointestinal tract during human digestion [52–55]. Dinoflagellates are distributed worldwide, and plankton-feeding animals such as bivalves feed on them. When dinoflagellate blooms, these animals accumulate OAs that become toxic, and consumption of them may cause DSP [36]. Likewise, the benthic dinoflagellate *Prorocentrum lima* has been identified as a producer of OAs [47]. In addition to their diarrheagenicity [56], the health risks for DSPs are further aggravated by the potential tumor-promoting activity of OAs [57]. DSP is a global issue for both public health and the shellfish industry. Unlike fish farming, bivalve farming does not impose any significant burden on the environment due to feeding and can be performed with relatively simple facilities and techniques, which explains its rapid expansion. However, the presence of DSP toxins has also increased, and monitoring them has become a global issue. The extensive geographic prevalence, frequent incidence, and notable health hazards have established DSP as a highly urgent issue in seafood safety [57]. Three methods can assess the harmful effects of OAs: MBAs, cell bioassays typically on neuro-2a cells [17], and the PP inhibition assay based on the strong and specific action of OAs in inhibiting PP2A [38,39]. MBA has been replaced by the LC-MS/MS method, which quantifies OAs with high accuracy and is employed for regulatory inspections [58]. However, to evaluate hazardous effects, the method must use OA equivalents defined for each respective OA derived from mouse lethality measured through either intraperitoneal (OAip) or oral administration (OAor). Consequently, total OA equivalents are utilized to ascertain the compliance of shellfish under the current regulatory system (0.16 mg/kg OA equivalent in whole meat) [55,59].



	R1	R2	R3	R4
OA	OH	CH_3	H	H
DTX1	OH	CH_3	CH_3	H
DTX2	OH	H	H	CH_3
DTX3	Acyl	CH_3	CH_3	H

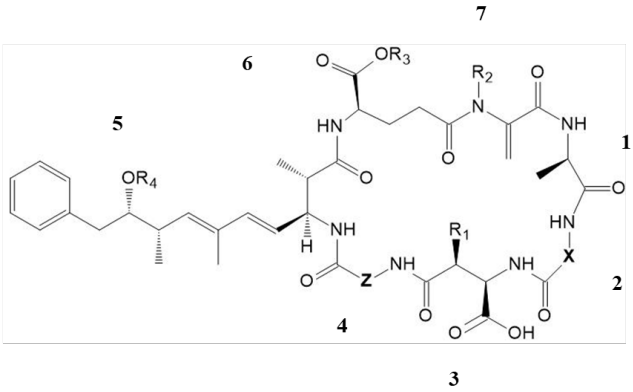
Figure 1. Chemical structure of okadaic acids (OAs).

3. Microcystins from Toxic Cyanobacteria

MCs are a group of cyclic heptapeptide hepatotoxins composed of five common amino acids and a pair of variable L-amino acids. The common chemical structure is cyclo-(D-alanine[1]-L-X[2]-D-MeAsp[3]-L-Z[4]-Adda[5]-D-Glu[6]-Mdha[7]), where MeAsp stands for *erythro*-β-methyl-aspartic acid, Mdha for *N*-methyldehydroalanine, Adda for 3-amino-9-methyl-2,6,8-trimethyl-10-phenyldeca- 4,6-dienoic acid, and X and Z denote two amino acids with variable identities [60]. Variations in structure have been reported for each of the seven amino acids, with the most common alterations involving demethylation at positions 3 and 7, as well as the replacement of L-amino acids at positions 2 and 4 (designated as X and Z) [60,61]. For example, MC-LR contains the amino acids leucine (L) and arginine (R) at these positions (Figure 2). MCs are produced by toxic cyanobacteria (blue–green algae) such as *Microcystis* and others [60]. *Microcystis aeruginosa* is the most common and widespread cyanobacterial species found in freshwater environments [62]. MC-LR is the representative and the most toxic MC variant; recent research has reported an increasing frequency of MC-producing *M. aeruginosa* blooms with climate change [63]. To date, ≥270 structural MC variants have been characterized from bloom samples and cultured strains of cyanobacteria [60,61,64,65]. Almost all MCs are highly toxic to aquatic organisms, wildlife, livestock, and humans, among others. Some intoxication events have been reported due to the ingestion of MC-contaminated water, including human poisoning [66], livestock poisoning [67], and mass mortality of wildlife [68]. For instance, the death of >50 patients with renal dysfunction in Caruaru, Brazil, was linked to the presence of MCs in water used for hemodialysis [66,69]. Accordingly, the World Health Organization has recommended a maximum allowable level of 1 μg/L MC-LR or its equivalent in water [70]. There are various detection methods for MCs in water [71]: biological (MBA), biochemical (PP2A inhibition assay and ELISA), and chemical methods (LC-MS, high-performance capillary electrophoresis, and gas chromatography), as well as novel biosensors. The traditionally used MBA, as a qualitative method to detect MC toxicity present in samples, is not an appropriate technique because of its lack of accuracy, sensitivity, and regard for animal welfare. High-performance liquid chromatography and LC-MS methods, developed as an alternative to MBA, are used for the quantification and identification of known MCs [2,3]. Recently, fast detection strategies of cyanobacterial blooms and associated cyanotoxins combining remote/proximal sensing technology with analytical/biotechnological analyses have been developed [72,73]. These methods require the reference materials of all MC analogs to quantify and/or identify. However, only a few are commercially available, namely MC-LR, -RR, -YR, -LF, and -LW. As a concrete example, in a previous study focused on monitoring MCs in environmental water resources using LC-MS, we needed to isolate and identify some MC variants from cultured strains and bloom samples of cyanobacteria

before analysis due to the lack of commercially available reference material [74]. On the other hand, the PP2A inhibition and ELISA assays are used for the rapid detection and quantification of total MCs using only one reference material of the representative analog. In addition, as a sample for the PP2A inhibition assay, health foods made of cyanobacterial products have appeared on the market. Many of these products are sold in the form of tablets, powders, or capsules. There exists a possibility for the products to be contaminated with microcystins because, in some cases, cyanobacteria are harvested from open water such as a lake [75–77]. The existence of MCs in food supplements containing spirulina, which were sold in the United States, was confirmed, and the Oregon Department of Agriculture, in 1997, established a safe threshold for MCs in blue–green algae supplements at 1 μg/g [78]. More than 100 samples of cyanobacterial products have been detected by different methods, including a PP2A assay which analyzed and reported contaminations of MCs [77]. The application of the PP2A inhibition method using rhPP2A should also be evaluated for these samples.

(a)



(b)

	1	2	3	4	5	6	7
MC-LR	cyclo-(D-Ala-	-L-Leu-	-D-MeAsp-	-L-Arg-	-Adda-	-D-Glu-	-Mdha)
[Dha^7]MC-YR	cyclo-(D-Ala-	-L-Tyr-	-D-MeAsp-	-L-Arg-	-Adda-	-D-Glu-	-Dha)
[D-Asp^3]MC-RR	cyclo-(D-Ala-	-L-Arg	-D-Asp	-L-Arg-	-Adda-	-D-Glu-	-Mdha)
[D-Asp^3]MC-HtyR	cyclo-(D-Ala-	-L-Hty	-D-Asp	-L-Arg-	-Adda-	-D-Glu-	-Mdha)
[D-Asp^3, Dha^7]MC-LR	cyclo-(D-Ala-	-L-Leu	-D-Asp	-L-Arg-	-Adda-	-D-Glu-	-Dha)
[D-Asp^3, (*E*)-Dhb^7]MC-LR	cyclo-(D-Ala-	-L-Leu	-D-Asp	-L-Arg-	-Adda-	-D-Glu-	-(*E*)-Dhb)
[D-Asp^3, (*Z*)-Dhb^7]MC-LR	cyclo-(D-Ala-	-L-Leu	-D-Asp	-L-Arg-	-Adda-	-D-Glu-	-(*Z*)-Dhb)
[D-Asp^3, (*E*)-Dhb^7]MC-HtyR	cyclo-(D-Ala-	-L-Hty	-D-Asp	-L-Arg-	-Adda-	-D-Glu-	-(*E*)-Dhb)
[D-Asp^3, (*Z*)-Dhb^7]MC-HtyR	cyclo-(D-Ala-	-L-Hty	-D-Asp	-L-Arg-	-Adda-	-D-Glu-	-(*Z*)-Dhb)
[6-(*Z*)-Adda^5]MC-RR	cyclo-(D-Ala-	-L-Arg-	-D-MeAsp	-L-Arg-	-*6-(Z)*-Adda-	-D-Glu-	-Mdha)

Figure 2. Chemical structure of MCs. (**a**) General structure of MCs. R_1 and R_2 are H or CH_3; R_3 = H, CH_3, or C_3H_6OH; R_4 = H, CH_3, or $COCH_3$; X and Z are variable L-amino acids. (**b**) Variable chemical groups in MCs. Adda = (*2S*,*3S*,*8S*,*9S*)-3-amino-9-methoxy-2,6,8-trimethl-10-phenyldeca-4,6-dienoic acid; d-MeAsp = *erythro*-β-methyl-d-aspartic acid; Dha = dehydroalanine; Mdha = *N*-methyldehydroalanine; Dhb = dehydrobutyrine; Mdhb = *N*-methylhydrobutyrine; (6Z) Adda = stereoisomer of the Δ^6 double bond; Hty = homotyrosine.

4. Structure and Function of PP2A and Inhibition by Natural Toxins

PP2A is a major enzyme in eukaryotic cells that dephosphorylates the serine/threonine residues of proteins and is one of the four major classes (PP1, PP2A, PP2B, and PP2C) of eukaryotic serine/threonine phosphoprotein phosphatases [26,79]. PP2A exists as a holoenzyme and contains a core dimer ($PP2A_D$) consisting of a highly conserved 36 kDa catalytic subunit (PP2Ac) and a 65 kDa scaffold A subunit (PR65/A). This dimer can constitute the holoenzyme by associating with a third, variable, regulatory B subunit divided into four families of unrelated proteins: PR55/B [80], PR61/B′ [81], PR72/B″ [82], and PR93 or PR110/B‴ [83] (Figure 3). Regulatory B subunits control the enzymatic activity and substrate specificity of PP2A, and the intercellular localization and tissue specificity of the holoenzyme [29,84]. The regulatory mechanisms of PP2A holoenzyme formation and pharmacological PP2A activation in cancer therapy have been well studied [85–88], and the noncatalytic function of PP2A as a contribution of PP2A to chromosome assembly is also known [89]. Several natural toxins and antibiotics, including OAs and MCs, inhibit PP2A [19,35–37,40–44]. The overall architecture of the PP2A core enzyme bound to OA and MC-LR and the PP2A holoenzyme was revealed by its crystal structure, revealing that despite their different chemical structures (Figures 1 and 2), OA and MC-LR bind to the same surface pocket on PP2Ac [90–93]. The methylation of Dha (dehydroalanine)

at position 7 in MC-LR is important for PP2A inhibition. Similarly, cys269 in PP2Ac and Mdha (*N*-methyldehydroalanine) at position 7 in MCs play crucial roles as interactive sites between PP2A and MCs [18]. Structurally, OA, DTX1, and DTX2 differ in the number and position of a methyl group (Figure 1). DTX-1 has a 35-methyl group in the equatorial position, whereas DTX-2 has a 35-methyl group in the axial position [45,94]. Molecular modeling indicated that the axial 35-methyl in DTX-2 may cause unfavorable interactions in the PP2A binding site, explaining its lower toxicity [95]. The methyl group at the hydrophobic end of OA is accommodated in the hydrophobic cage in PP2Ac, one end of the binding pocket [92]. Therefore, the different inhibitory potency of OAs on PP2A might be due to differences in the number and position of the methyl group in the backbone structure of OAs, which might be important for PP2A inhibition similar to that in MCs. These possibilities have provided insights into the structural basis for understanding PP2A holoenzyme assembly, substrate recruitment, inhibition by natural toxins, and regulation by phosphorylation and methylation.

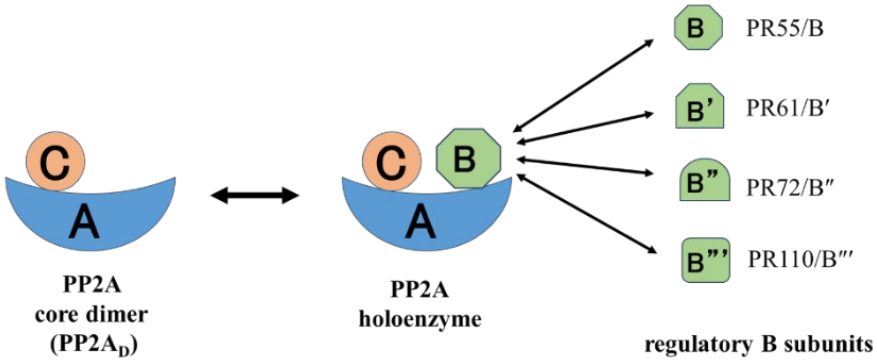


Figure 3. PP2A core dimer (PP2A$_D$) and heterotrimeric holoenzymes. One scaffold A subunit (A) binds to one catalytic subunit (PP2Ac) (C) to form an A/C core dimer (PP2A$_D$). This dimer can constitute the holoenzyme by associating with a third, variable, regulatory B subunit (B), comprising four families of unrelated proteins: PR55/B, PR61/B′, PR72/B″, and PR93 or PR110/B‴.

5. PP2A Inhibition Assay for Detection of OAs and MCs

Based on the specific and strong PP2A inhibitory action of OAs and MCs, a colorimetric PP2A inhibition assay using *p*-NPP as a substrate was developed [11,20–25,38,39]. PP2A hydrolyzes *p*-NPP (colorless) and produces *p*-NP (yellow) (Figure 4). Figure 5a shows an example image from the PP2A inhibition assay performed on a 96-well microplate. The color intensity is proportional to the enzyme activity, and the absorbance was measured at 405 nm. An example of a dose-dependent curve constructed from the absorbance date is shown in Figure 5b.

The concentration of these toxins in samples can be calculated from the calibration curve prepared using the respective reference materials of the toxin classes. In early studies, colorimetric PP2A inhibition assays employed a native PP2A extracted from human hepatocytes, human red blood cells [22], or rabbit skeletal muscle [23]. However, this approach was not widely applied because of fluctuations in enzyme quality, given the importance of having a PP2A product of high purity and good stability for practical use. Although several recombinant protein expression systems, such as bacterial, yeast [96,97], and mammalian [98,99] expression, had been used to produce an active recombinant PP2Ac (rPP2Ac) at high yield, these systems did not produce high-quality PP2A. For use in assay kits, genetic engineering techniques employing the baculovirus expression system and High Five insect cells can be utilized to generate active rPP2A [100,101]. Furthermore, an optimized methodology was introduced for the high-yield production of rPP2Ac with high activity that is achieved at a lower temperature (19 °C) compared to the conventional culture conditions (27 °C), employing the baculovirus expression system [101], and its suitability for use in a microplate assay was evaluated previously [21,25,102]. The recombinant human

PP2A catalytic subunit (rhPP2Ac) was purified in a simple step with good reproducibility (Figure 6a). Recombinant baculoviruses encoding $His_{8\times}$-tagged human PP2Ac (vHis-PP2Ac) were produced using the Bac-to-Bac baculovirus expression system (Invitrogen) according to the manufacturer's protocols and amplified using Sf9 (*Spodoptera frugiperda*) cells. To obtain active recombinant PP2A, $His_{8\times}$-tagged rhPP2Ac was expressed in High Five insect cells and purified from cell lysates using ethanol precipitation and affinity purification with Ni–NTA agarose, as shown in Figure 6 [100,101].

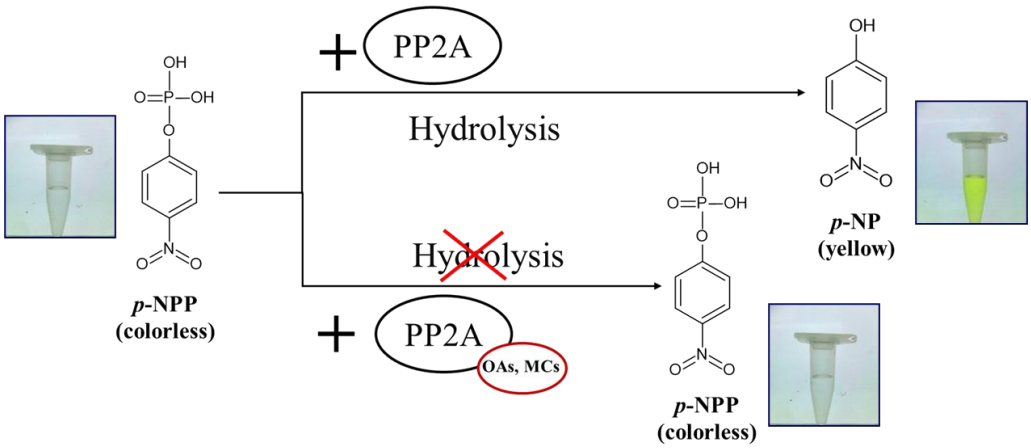


Figure 4. Principle of the PP2A inhibition assay. PP2A can hydrolyze a colorless artificial substrate, *p*-nitrophenyl phosphate (*p*-NPP), and produce *p*-nitrophenol (*p*-NP), which is yellow.

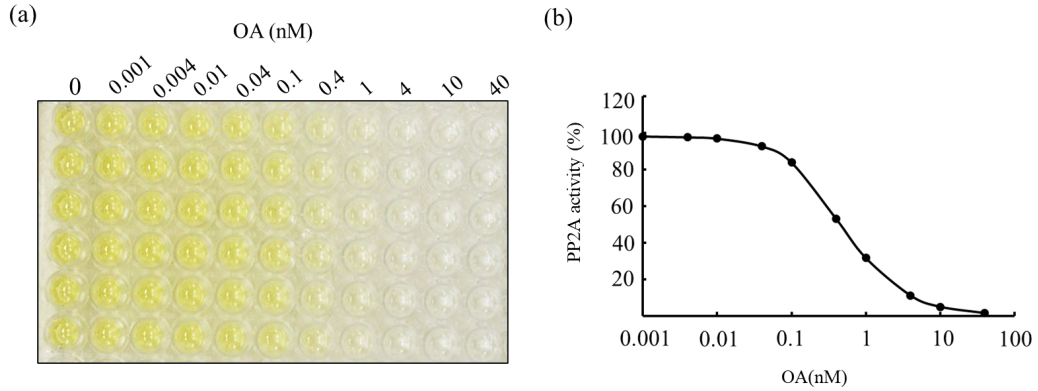


Figure 5. Examples of images obtained with the PP2A inhibition assay. (**a**) Color intensity proportional to enzyme activity on a 96-well microplate. (**b**) Dose-dependent curve for PP2A inhibition by OA (okadaic acid). Each point represents the mean (n = 6).

Using rhPP2Ac and *p*-NPP, assay kits for the rapid detection of OAs and MCs were developed [21,25,103]. In the kits, a calibration curve for quantification can be created using OA and MC-LR as standard substances. This curve can be utilized to calculate and express the concentrations of OA and MC in the samples as OA or MC-LR equivalents. For OAs, the measurable range in the PP2A inhibition assay was determined based on the calibration curve established using the certified reference material (CRM) of OA, as shown in Figure 7.

Matrix blanks, both unhydrolyzed and hydrolyzed, were prepared using mussel and scallop extracts devoid of OAs to determine the limit of detection (LOD) and the limit of quantification (LOQ) for OA detection [16,25,102]. The LOD was estimated as the sum of the average of matrix blank values and three times the value of the standard deviation (SD, 3xSD), and the LOQ was estimated as the sum of the average of matrix blank values and 10 times the value of SD (10xSD; Table 1). As officially recommended, a hydrolysis step is required to detect the presence of DTX3 [55] because this very weak PP2A inhibitor [25,104,105] can only be detected in the PP inhibition assay as free OAs with an alkaline hydrolysis step. For

MCs, the assay can detect MC-LR in water at 0.005–5 ng/mL [21]. These values are below the WHO-recommended level of 1 μg/L; the water sample could be directly applied to the assay without concentration.

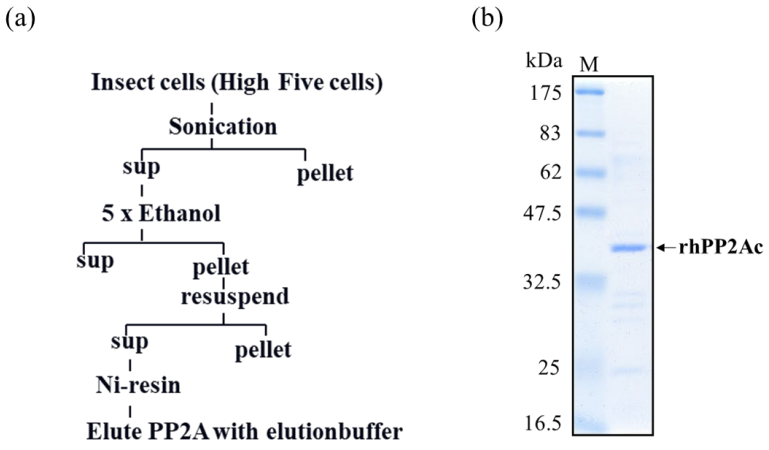


Figure 6. Purification scheme of recombinant human PP2A catalytic subunit (rhPP2Ac). (**a**) Recombinant human PP2Ac (rhPP2Ac) was synthesized in High Five insect cells by infection of recombinant baculovirus encoding $His_{8\times}$-tagged human PP2Ac and purified from cell lysates using ethanol precipitation and affinity purification with Ni–NTA agarose. (**b**) After purification, the recombinant protein was subjected to 12% SDS–PAGE and visualized using Coomassie brilliant blue R staining according to the method described in the reference [101].

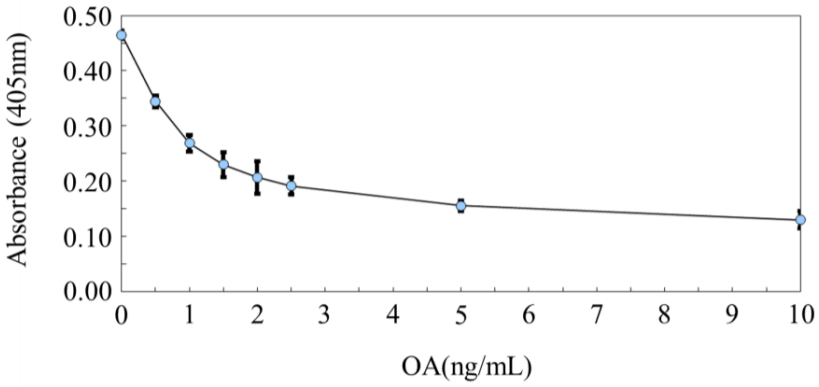


Figure 7. Calibration curve using the net absorbance of OA standard solutions. The PP2A inhibition assay was performed using OA standard solutions (0, 0.5, 1.0, 1.5, 2.0, 2.5, 5.0, and 10.0 ng/mL) prepared from OA CRM (CRM-OA-d, NRC Canada) according to the method described in the reference [16]. Each point in the calibration curve represents the mean (n = 3) with an RSD of 2.5%.

Table 1. Limits of detection (LOD) and quantitation (LOQ) for okadaic acids in shellfish using PP2A inhibition assay.

Matrix	LOD (μg/g)	LOQ (μg/g)	Reference
Mussels			
Unhydrolyzed	0.0387	0.0765	[16]
	0.0424	0.0725	[25]
Hydrolyzed	0.0646	0.0989	[16]
	0.0476	0.0932	[25]
Scallops			
Unhydrolyzed	0.0217	0.0372	[25]
	0.0262	0.0470	[102]
Hydrolyzed	0.0274	0.0415	[25]
	0.0432	0.0780	[102]

The current regulatory level of OAs is 0.16 mg/kg OA equivalent in the whole meat of bivalve [55,59], and the guidance level of MCs in water is 1 µg/L MC-LR or its equivalent [70]. The concentration of each analog obtained using instrumental analysis, such as LC-MS, should be converted into OA or MC-LR equivalents using toxicity equivalency factors (TEFs). The 50% inhibitory concentration (IC_{50}) values of each OA and MC analog for inhibiting rhPP2Ac provides the OA equivalent (Oapp2a) and MC-LR equivalent, respectively (Table 2). The relative ratios (OApp2a) for OAs [16] and conversion factors for MCs [18], based on the IC_{50} values, allow for the calculation of the total OAs or MCs concentration in the samples as the OA equivalent and MC-LR equivalent, respectively, when using the instrumental analysis method. The assay kits used OA or MC-LR as standards and quantified the total OAs or MCs concentration in the samples, as OA or MC-LR equivalents, respectively. Compared to instrumental analysis, the PP2A inhibition assay has the following advantages: no need to prepare all analog reference materials, toxicity evaluation not requiring TEF as it uses OA or MC-LR as standards, ready to use as a kit, and adaptability to both OAs and MCs by simply changing the reference material. So far, we have evaluated the PP2A inhibition assay for the detection of OAs in the whole meat and digestive gland of bivalves and of MCs in environmental waters.

Table 2. IC_{50} values of okadaic acids (OAs) and microcystins (MCs) in the PP2A inhibition assay.

Toxins	PP2A Inhibition		Reference
OAs	**IC_{50} (nM)**	**OApp2a [a]**	
OA	0.14	1	[16]
DTX1	0.09	1.6	[16]
DTX2	0.45	0.3	[16]
MCs	**IC_{50} (nM)**	**Conversion factor [b]**	
MC-LR	0.032	1.000	[18]
MC-RR	0.056	0.571	[18]
MC-FR	0.069	0.464	[18]
MC-LF	0.096	0.333	[18]
[D-Asp^3]MC-HtyR	0.098	0.327	[18]
[D-Asp^3, (Z)-Dhb^7]MC-HtyR	0.110	0.291	[18]
MC-LW	0.114	0.281	[18]
[D-Asp^3, (E)-Dhb^7]MC-HtyR	0.122	0.262	[18]
MC-YR	0.125	0.256	[18]
MC-LA	0.161	0.199	[18]
[D-Asp^3, (Z)-Dhb^7]MC-LR	0.164	0.195	[18]
[Dha7]MC-LR	0.167	0.192	[18]
MC-WR	0.179	0.179	[18]
[D-Asp^3, (E)-Dhb^7]MC-LR	0.201	0.159	[18]
[D-Asp^3, Dha^7]MC-RR	0.220	0.145	[18]
[D-Asp^3, Dha^7]MC-LR	0.254	0.126	[18]
[Dha^7]MC-RR	0.293	0.109	[18]
[D-Asp^3]MC-RR	0.300	0.107	[18]
[Dha^7]MC-YR	0.379	0.084	[18]
Nodularin	0.540	0.059	[18]
[6-(Z)-Adda^5]MC-RR	10.126	0.003	[18]

[a] OA equivalent based on PP2A inhibition activity. [b] MC-LR equivalent based on PP2A inhibition activity.

6. Conclusions

This review summarized the principles and uses of a PP2A inhibition assay for the rapid detection of OAs and MCs. To manage the health risk posed by these toxins, rapid, sensitive, and inexpensive methods to monitor the levels of OAs in shellfish and of MCs in aquatic environments or drinking water are urgently needed. Since these toxins strongly and specifically inhibit PP2A, they can be used to assay MCs and OAs. The method is simple, cost-effective, field-ready, and suitable for processing numerous samples, making it

a viable replacement for MBA. Additionally, combining the PP2A inhibition assay with LC-MS analysis offers an efficient monitoring approach for rapidly assessing the environmental and health risks associated with hepatotoxic cyanobacteria producing MCs and toxic dinoflagellates producing OAs [74,106]. HPLC and LC-MS are employed for quantifying and identifying known OAs and MCs through instrumental analysis, whereas the protein PP2A inhibition assay is utilized for the swift detection and quantification of the total content of these toxins. PP2A not only plays a pivotal role as a protein phosphatase in important cellular events but also serves as a valuable tool in the rapid detection of natural toxins such as OAs and MCs.

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